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Research Article

The Usefulness of Diagnostic Vitrectomy in Neoplastic and Non-Neoplastic Masquerade Syndrome: An Observational Study

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Abstract

Introduction: Masquerade intraocular inflammation may be considered neoplastic or non- neoplastic masquerades such as primary intraocular lymphoma, leukemia, infectious and inflammatory diseases. These pathologies require a definitive diagnosis, as the treatment modalities are different. The aim of our study was to investigate the safety and usefulness of diagnostic vitrectomy with vitreous humor flow cytometry in eyes with intraocular inflammation of unknown etiology.

Methods: A retrospective observational study included 35 eyes of 29 patients with atypical intraocular inflammation unresponsive to corticosteroid therapy. In all cases diagnostic vitrectomy with flow cytometry analysis of the vitreous specimen was performed.

Results: Among 35 eyes, the result of diagnostic vitrectomy analysis showed unspecific inflammatory response in 7 (20.0%) eyes, confirmed neoplastic diseases in 5 (14.3%) eyes. All of them it was intraocular lymphoma but one of the eyes with primarily diagnosed lymphoma and one of the eyes with primarily diagnosed unspecific inflammatory response in flow cytometry has been diagnosed finally as a choroidal melanoma after enucleation of the eyeball. Diagnostic vitrectomy excluded neoplastic disease in 7 eyes (20.0%). In 3 eyes (8.6%) bacterial infection, in 4 eyes (11.4%) viral infection. In 2 eyes (5.7%) we excluded bacterial infection, in 7 cases (20.0%) no conclusive results were obtained. The most common adverse event was cataract in patients (12 eyes, 34.3%).

Conclusion: Diagnostic vitrectomy with flow cytometry of vitreous humor is helpful in confirming the clinical suspected diagnosis of posterior segment inflammation. Flow cytometry need to be complemented with other diagnostic test including cytopathology, especially in cases suspected of intraocular lymphoma. Flow cytometry of the vitreous humor in choroidal melanoma is not a useful diagnostic tool.

Keywords: Diagnostic vitrectomy; Flow cytometry; Intraocular inflammation; Intraocular lymphoma

Introduction

Intraocular inflammation of the posterior segment of the eye may be associated with ocular masquerade syndrome due to neoplastic, infectious and inflammatory diseases [1-3]. In most cases, the clinical features are so characteristic that performing standard other diagnostic tests allows confirming the diagnosis and implementing of appropriate treatment.

In cases where the clinical picture is not characteristic and cannot be diagnosed, and when therapy with steroids is not effective, performing diagnostic vitrectomy with the use of flow cytometry may be an additional diagnostic tool [4]. This is particularly important in conditions that potentially are sight threatening or even mortal such as vitreoretinal or choroidal lymphoma [5]. In those cases, a valuable result is also the exclusion of the presence of lymphoma cells in the vitreous specimen tested. In addition, biopsy of retinal and choroidal lesions can be obtained during diagnostic vitrectomy after vitreous sampling [8]. The aim of our study was to assess the safety of diagnostic vitrectomy and the effectiveness of confirming clinical diagnosis in cases of atypical or not responding to empiric treatment intraocular inflammation and lesions suspected of being a neoplastic process.

Materials and Methods

We performed an observational, retrospective study of 35 eyes of 29 patient who underwent 23G diagnostic vitrectomy at the another unit between the years 2013-2019. The patients were divided into the following groups according to the suspected diagnosis: idiopathic posterior uveitis unresponsive to corticosteroid treatment 15 eyes (42.9%), suspected neoplastic disease 15 eyes (42.9%), viral retinitis 2 eyes (5.7%) and patient with other intraocular lesion with haemophtalmus or endogenous endophthalmitis 3 eyes (8.6%) (Table

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Table 1: Results after diagnostic vitrectomy.

Suspected Diagnosis	Number of eyes	Unspecific inflammatory response	Confirmed neoplastic disease	Viral infection	Bacterial infection	Excluded neoplastic disease	Excluded bacterial infection	No conclusive result
Idiopathic posteriori uveitis	15	4	2	2	3	0	1	3
Neoplastic disease	15	2	3	0	0	7	0	3
Viral retinitis	2	0	0	2	0	0	0	0
Other intraocular changes (endogenous, endophtalmitis, hemophtalmus)	3	1	0	0	0	0	1	1

1). Patients included in the study underwent serum testing for all known causes of posterior uveitis (tuberculosis, syphilis, HIV, HSV, CMV, VZV, *Toxoplasma gondii*) which did not allow to establish a definitive diagnosis [2,4,5]. Two-three weeks before vitrectomy the steroids were stopped. Patients were evaluated preoperatively, on postoperative day one, after six weeks and six month follow up. Preoperative clinical data was collected from each patient including age and sex. Ophthalmic data included findings obtained on clinical examination, which included Best Corrected Visual Acuity (BCVA) assessed with Snellen charts, Intraocular Pressure (IOP), anterior and posterior segment assessment, ultrasonography A- and B-scan and in selected cases Fluorescein Angiography (FA). The follow-up examinations were performed one day postoperatively, then 1 week, 1 month and 6 month after surgery and then the follow-ups depended on the clinical status of the patient and included the same parameters as the baseline tests except for FA. In all cases, vitreous specimens underwent the following testing: microbiological culture, PCR test for viruses (HSV-DNA, CMV-DNA, VZV-DNA) and *Toxoplasma gondii* and cytofluorometric analysis with flow cytometry. The vitreous specimens for analysis were sent to laboratory immediately after taken. In patients with positive serum tests for tuberculosis (QuantiFeron Tbc Gold test), a vitreous examination was performed for the presence of tuberculosis mycobacteria DNA using a genetic probe (Becton Dickinson). The results of diagnostic vitrectomy were divided into the following groups: unspecific inflammatory response, confirmed neoplastic disease, viral infection, bacterial infection, excluded neoplastic disease, excluded bacterial infection and no conclusive results (Table 1).

Surgical technique

In all patients, a 23G diagnostic vitrectomy was performed [6-8]. 23-gauge trocars cannulas were placed 3.5-4.0 mm from the limbus. Non-diluted vitreous was excised with a vitrectomy cutter and aspirated into a 5cc syringe manually. The eye was filled with air to prevent hypotony, to obtain undiluted vitreous specimen and to prevent the risk of reducing the specimen cellularity. After collection of 2-3 ml of undiluted vitreous the infusion was changed into fluid and vitrectomy was completed. If necessary, in 7 cases, retinal or choroidal lesions biopsy was taken and sent for cytological examinations. After the surgery, patients were treated with antibiotics and steroids eye-drops.

Flow cytometry analysis

The leukocytes subpopulations were assessed by flow cytometry in vitreous humor specimens of 35 eyes. Samples were collected on a flow cytometer FACS Canto II (BD Biosciences), and analyzed using BD FACS Diva software v 6.1.2.

Antibodies used for the study

Monoclonal antibodies were used to analyze leukocytes. Antibodies were titrated to determine optimal concentrations. Antibody-capture beads (CompBeads, BD Biosciences) were used for single-color compensation controls for each reagent used in the study. The panel of antibodies was the base for detecting the cell populations from lymphocyte lineage (T, B and NK). Standard panel that was used in this study: for T-lineage: anti- CD3, CD4, CD8, CD2, CD7, CD5, HLADR; for B-lineage: anti - CD19, CD20, CD22, CD23, CD43, CD10, kappa and lambda light chains; for NK cells anti-CD56, CD16. The myelomonocytic lineage cell was identified by demonstrating expression of antigens: CD14, CD4, CD16, CD64, CD33, CD13, CD15, CD56 and HLADR.

Staining protocol

Routinely for the testing, a minimum of 150ul of the vitreous was used and incubated with a pre-mixed antibody cocktail for 15 minutes in the dark at room temperature. After staining, admixture erythrocytes were lysed for 2min, using cell lyse solution (BD FACS Lysing Solution, BD Biosciences-diluted 10x with deionized water). Next cells were washed once, with 2ml of PBS containing 2% FBS, and finally resuspended with 0.5ml PBS. We collected, from each tube, between 0.5×10^4 and 1.0×10^4 events per sample. Eight colour FMC analysis was performed on all samples using FACS Canto II (Becton Dickinson).

Results

Thirty-five eyes of 29 consecutive patients were included in this study (Table 1). There were 12 men (41.4%) and 17 (58.6%) woman. Mean patient age was 58.3 ± 18.5 years (range 20-90). Mean preoperative BCVA was 20/2222 (0.009 ± 0.052, 2.03 ± 1.28 LogMAR). The mean follow-up time was 26.1 ± 14.4 months (range: 6-52 months). The slit-lamp examination revealed the signs of anterior chamber inflammation in 12 eyes (34.3%), 20 eyes (57.1%) were pseudophakic. The fundus copy showed the presence of vitersous haze and inflammatory cells in 18 eyes (51.4%). Five eyes (14.3%) showed evidence of yellowish- whitish subretinalinfiltrates indicative for intraocular lymphoma. The A- and B-scan ultrasongraphy showed the presence of non-specific subretinal infiltrations, mass in 5 eyes (14.3%).

In 15 eyes (42.8%), diagnostic vitrectomy was performed for idiopathic posterior uveitis unresponsive to conventional corticosteroid therapy, in 15 eyes for suspected neoplastic disease (42.8%), in 2 eyes (5.7%) for suspected viral retinitis sand in another 3 eyes for other reasons such as intraocular lesion with hemophtalmus or endogenous endophthalmitis (8.6%). In six patients, the diagnostic vitrectomy was performed in both eyes, which were suspected of

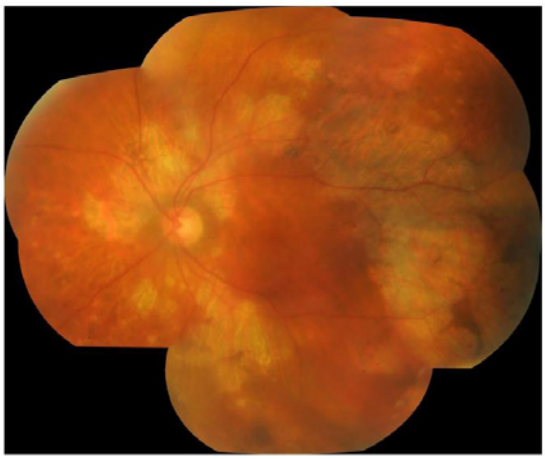

Figure 1A:

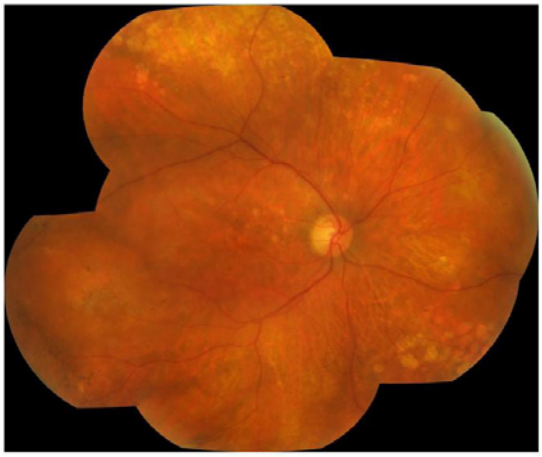

Figure 1B:

bilateral intraocular inflammation or lymphoma. No intraoperative complications were noted in our group of patients.

The most common given result of diagnostic vitrectomy was unspecific inflammatory reaction in 7 eyes (20.0%), followed by excluding suspected neoplastic disease in 7 eyes (20.0%). In 5 eyes (14.3%) the result of flow cytometry was indicative of neoplastic disease. In all 5 cases of the neoplastic disease, lymphoma was suspected clinically. In 4 eyes of 2 patient the flow cytometry showed the presence of monoclonal proliferation of atypical B-cell lymphocytes which confirmed the diagnosis of B-cell intraocular lymphoma. One patient showed the immunophenotypic features that correspond to the T-cell lymphoma (T-cells lymphocyte = 86.6% of population, CD4/CD8 = 7.3 may correspond to Non-Hodgkin lymphoma (NHL-T)). The patient was referred to a hematologist who diagnosed isolated intraocular lymphoma and introduced treatment. Due to the lack of response to therapy and deterioration of the local condition, enucleation was performed and definitive diagnosis of malignant melanoma was made. In 2 cases (5.7%) the vitreous specimen analysis with PCR test was positive for viral infections (HSV) and in 3 other cases (8.6%) the specimen culturing was positive for bacterial infection (*Enterococcus fecalis*). The most common viral pathogen was HSV-1 identify in PCR test.

In the group of patients who underwent diagnostic vitrectomy due to idiopathic uveitis (15 eyes) in 12 eyes (80.0%) flow cytometry gave conclusive diagnosis. In 4 eyes (26.7%) of this group it was unspecific inflammatory reaction. In 2 eyes (13.3%) diagnostic vitrectomy confirmed neoplastic disease, it was intraocular B-cell lymphoma (monoclonal growth of B cell lines with the antigen pattern CD19+/CD22+/HLA- DR+/KAPPA+/CD38+/CD56+). In 2 eyes (13.3%) diagnostic vitrectomy after PCR analysis confirmed viral infection, in 3 eyes (20.0%) specimen culturing was positive for bacterial infection. Diagnostic vitrectomy excluded bacterial infection in 1 eye (6.7%) and there was no eyes with excluded neoplastic disease in those group of eyes. In 3 eyes (20.0%) diagnostic vitrectomy gave no conclusive results. In 1 of 4 eyes of the group with unspecific inflammatory reaction choroidal melanoma after enucleation was diagnosed.

In-group of suspected neoplastic disease (15 eyes) diagnostic vitrectomy gave conclusive diagnosis in 12 eyes (80.0%) in 7 eyes (46.7%) it helped excluded neoplastic disease and confirm in 3 eyes (20.0%). In all of these 3 eyes, lymphoma (Figure 1A and 1B) was diagnosed although at a later time 1 of them turned out to be malignant melanoma. In 2 eyes (13.3%) in which neoplastic disease was suspected, it was possible to diagnose inflammatory reaction. In 3 (20.0%) out of 15 cases suspected of neoplastic disease flow cytometry results were not obtained.

In-group of viral retinitis all cases (100%) of suspected viral retinitis (2 eyes) after diagnostic vitrectomy confirmed preoperative diagnosis.

In the group of other intraocular changes (3 eyes) 1 eye (6.7%) was unspecific inflammatory reaction, 1 eye (6.7%) was excluded bacterial infection and 1 eye (6.7%) was no conclusive diagnosis.

In all of the cases in 7 eyes (20%) no flow cytometry results was obtained due to low cellularity of the sample. In cases of undefined inflammatory reaction in 2 cases we were able to get final diagnosis. In 1 cases because of deteriorating state of the eye and negative results of vitrectomy we decided to perform enucleation and after histopathology testing choroid melanoma was diagnosed. In other case after few weeks after diagnostic vitrectomy we received positive result of quantiferon test for tuberculosis. In other cases of undefined inflammatory reaction, we were not able to give final diagnosis.

Mean postoperative BCVA was 20/571 (0.035 ± 0.088, 1.46 ± 1.05 LogMAR) assessed one month after surgery. Best corrected visual acuity remained stable in 21 eyes (60%), improved in 14 eyes (40%). Most common postoperative adverse event was cataract in 12 eyes (34%). Intraocular pressure elevation was observed in 3 eyes. One patient had intraocular pressure raised preoperatively, only in 2 eyes (n=5.7%) postoperative intraocular pressure was raised over 25mmHg in early postoperative stages. No other postoperative complications were observed in study group during observation period. In one case, postoperative haemopthalmus was observed.

Discussion

Effective treatment of eye diseases, especially those that are sight- or even life- threatening may cause the loss of vision or life, like intraocular lymphoma must be based on accurate diagnosis.

Delayed or inappropriate treatment due to misdiagnosis seriously reduces therapeutic success. In order to make the right diagnosis and at the same time to treat the patient surgically, we decided to assess the efficacy and safety of diagnostic vitrectomy. The main objective of the study was to assess the possibility of making a diagnosis and its compliance with clinical diagnosis and safety assessment after using diagnostic vitrectomy followed by microbiological, cytological, PCR and with flow cytometry analysis of the vitreous specimen.

Our group of patients treated in our clinic underwent thorough diagnostics appropriate to clinical suspicions similarly to other authors [6,9]. We included those patients who underwent a vitrectomy with flow cytometry for our study. In our patients, there were eyes with posterior segment inflammation, in whom systemic treatment with steroids was not effective. Patients with suspected infectious viral diseases, neoplastic and other diseases constituted the next group. In addition to flow cytometry, we performed other tests of the collected material, including culture for growth of bacterial or fungal organism, cytological examination, PCR for detection of genomic micro organismal DNA and testing with a genetic probe system to detect DNA of tuberculosis.

The most common results in flow cytometry was unspecific inflammatory response in 7 eyes (20.0%), followed by excluding suspected neoplastic disease in 7 eyes (20%) and no conclusive results in 7 eyes (20%). The results of other authors are difficult to compare with our because of the quite different group of patients. Maruyama et al. [10] divided patients into following groups: definitive or suspected sarcoidosis, intraocular tumor, viral infection, non- sarcoidosis and unknown etiology. Because of a large number of patients with malignant intraocular tumor treated in our department, decided to distinguish the group of eyes with excluded neoplastic disease. Exclusion of the neoplastic disease in as many as 20% of cases (35 all eyes, 15 eyes with suspected neoplastic diseases) was valuable diagnostic information. In 4 cases (11.5%) due to the flow cytometry results the neoplastic disease was confirmed. In all of these 4 eyes, intraocular lymphoma was diagnosed, although one of them based on the histopathological examination of the enucleated eye turned out to be malignant melanoma.

Cantu at al. [11] underlined that; the flow cytometry analysis must be combined with cytopathological testing to diagnose the vitreoretinal lymphoma. When analyzing the results of our study, we also strictly recommend carrying out a cytopathological test. The most common complications that may occur after pars plana vitrectomy surgery have been evaluated: cataract in phakic eyes, retinal detachment (iatrogenic), vitreous hemorrhage, endophtalmitis, hypertony or hypotony [7]. In our group of patients cataract was in 12 of phakic eyes, intraocular pressure changes were observed in 3 eyes. One patient had intraocular pressure raised preoperatively. Postoperative intraocular pressure was raised over 25mmHg only in 2 eyes (n=5.7%) in early stages after surgery. No other postoperative complications were observed in study group during observation period. In all of the cases in 7 eyes (20%) no flow cytometry results was obtained due to low cellularity of the sample. The reason for this may be the size of taken sample during surgery; sometimes-single diagnostic vitrectomy may miss abnormal cells we are interested in.

The results of our study showed that the diagnosis of intraocular lymphoma cannot be made only on the basis of flow cytometry. The diagnosis must be completed with a cytopathological examination. This was clearly demonstrated by the misdiagnose of lymphoma, which after the progression of the disease and enucleation of the eye was found to be a choroidal melanoma. Flow cytometry of the vitreous humor in choroidal melanoma is not a useful diagnostic tool. We must also remember that chronic corticosteroid therapy could have had a repressing effect on the tumor proliferation and differentiation [12]. The limitations of this study are related to the material sample size, heterogeneity of patients group and the lack of evaluation in the cytopathology test in all cases.

Conclusion

Diagnostic vitrectomy with flow cytometry analysis of vitreous humor in challenging cases of unknown etiology intraocular inflammation can be a safe and useful diagnostic tool. However, in cases suspected of intraocular lymphoma the flow cytometry need to be complemented with cytopathological testing. Flow cytometry of the vitreous humor in choroidal melanoma is not a useful diagnostic tool.

Summary Statement

To investigate the safety and usefulness of vitreous humor flow cytometry performed after diagnostic vitrectomy in eyes with chronic intraocular inflammation of unestablished etiology. Flow cytometry of vitreous humor cells may be a helpful diagnostic tool in confirming the diagnosis in atypical cases of intraocular inflammation. Observed intra- and postoperative complications were not significant.

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